II. REMARKS

Status of the claims

Claims 1-30, 34-51 and 53-56 are pending in this case, following election with traverse of Groups I and II in a communication filed on December 18, 1996 in response to the Restriction Requirement dated September 18, 1996. By virtue of this amendment, claims 31-33, 52 and 57-68 are canceled without prejudice. Claims 1-30, 34-51 and 53-56 have been examined and are rejected. These rejections are addressed in the appropriate sections below.

Generally, the claims are amended to define the invention more specifically and to correct errors pointed out by the Examiner or noted by the Applicants. In particular, claims were amended to provide proper antecedent basis for the recited language. The claim amendments are addressed in detail below.

The amendments to the claims are supported by the specification and no new matter has been introduced. Entry of these amendments is respectfully requested. Reexamination, reconsideration and allowance of the claims, as amended, are respectfully requested.

Summary of the invention

The present invention provides a method of specifically separating intact cells based on products secreted by the cells. The method generally involves coupling the surface of the cells to a capture moiety specific for the secreted product, culturing the cells under conditions in which the product is secreted, released and specifically bound to the capture moiety, then separating the cells on the basis of the bound product. Also provided is a method for labeling cells with a secreted product, generally involving the steps of coupling the surface of the cells to a capture moiety and culturing the cells under conditions in which the product is secreted and released. In this method, the product is captured by the capture moiety. The invention also provides a composition of matter comprising cells capable of capturing a product secreted and released by

the cells in which the surface of the cells is coupled to a capture moiety. The invention further provides cells separated by the disclosed separation method. Another aspect of the present invention is a kit for use in detection of cells that secrete a desired product. The kit includes a product capture system comprising at least one anchor moiety, at least one capture moiety and at least one label moiety. The invention further provides a method for identifying cells in a mixed population which secrete a product and separating them from the mixed population of cells.

Objections to the specification

Applicants acknowledge receipt of PTO Form 948. Formal drawings will be submitted upon allowance of the application.

The Examiner noted that the application does not contain an abstract of the disclosure. An Abstract is provided on the first page of the PCT publication.

The Examiner noted that, in order for an application to claim the benefit of priority of an earlier filed application, the later filed application must contain a specific reference to the earlier filed application. Applicants have amended the specification accordingly.

The Examiner noted the use of the trademarks SEPHADEX™, TWEEN™ and FICOLL™ and required that they be capitalized and accompanied by the generic terminology. Applicants have amended the specification accordingly.

Provisional obviousness-type double patenting rejection

Applicants note that Claims 1-13 and 53-56 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-6 of copending patent application Serial No. 08/441,259. Applicants will submit a terminal disclaimer upon issuance of a Notice of Allowance in either the 08/441,259 or the present application.

Rejections under 35 U.S.C. §112, first paragraph

Claims 1-30, 34-40, 43-50 and 53-56 are rejected under 35 U.S.C. §112, first paragraph on the ground that the specification does not provide enablement for the claimed method or kit which does not use high viscosity or gel forming media. This is an insufficient ground for rejection under 35 U.S.C. §112, first paragraph.

In order to establish and sustain a 35 U.S.C. §112, first paragraph rejection, the Examiner must establish doubt as to the truth of the assertion. This has not been provided; the burden has thus not passed to applicants to establish the truth of the asserted claim scope. Claims 22-28 are not drawn to a method or a kit, but to a composition of matter comprising cells having the recited characteristics. Since the issue of the use of high viscosity or gel forming media is not germane to claims 22-28, Applicants presume that inclusion of these claims in this rejection was unintentional.

Regarding claims 1-21, 29-30, 34-40 and 53-56, and notwithstanding the lack of requirement for examples, the specification provides ample instruction for the claimed method or kit which does not use high viscosity or gel-forming media. As shown in Example 1, Applicants have demonstrated that a mixed population of cells to which a capture antibody has been coupled can be separated on the basis of secretion of IgM. A mixture of biotinylated B.1.8 (among which are cells that are known to secrete IgM) and X63 Ag86.5.3 (which do not secrete IgM) cells was conjugated with capture antibodies. At various times after incubation, the cells were tested for the presence on the cell surface of captured IgM. The results shown in Figure 6b demonstrate that two populations were clearly distinguishable after a 30 minute incubation and could therefore be separated. Neither high viscosity nor gel-forming media were used in these experiments; nevertheless, Applicants demonstrated that cell separation was feasible. Applicants have therefore enabled claims 1-21, 29-30, 34-40 and 53-56.

Additionally, on page 18, lines 9-32 of the specification, applicants provide further instructions for a method not involving high viscosity conditions. Thus, in the absence of a proper showing of lack of enablement, the claims are patentable under 35 U.S.C. §112, first paragraph. Even if the proper degree of doubt had been established by the Patent Office, it would not be sustainable, since the specification provides ample examples to allow one of skill in the art to make and use the claimed invention.

Claims 1-30, 34-40, 43-50 and 53-56 are rejected under 35 U.S.C. §112, first paragraph, on the grounds that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims. Specifically, the Examiner states that "the specification, while being enabling for the claimed method or kit wherein antibody or gamma interferon secreting cells are detected, does not reasonably provide enablement for the claimed method or kit for the detection of cells secreting any molecule per se."

Applicants respectfully traverse. As noted above, claims 22-28 are not drawn to a method or a kit, but to a composition of matter comprising cells having the recited characteristics. Since claims 22-28 are drawn neither to a method nor a kit, their inclusion in this rejection was obviously unintentional.

Regarding claims 1-21, 29-30, 34-40 and 53-56, the Examiner has not presented convincing evidence that the claimed method and kit would not work for the detection of cells secreting molecules other than antibody or gamma interferon. To support his contention, the Examiner cites Weissman et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:1463-1466 (hereinafter "Weissman"). The Examiner notes that Weissman teaches that secreted interleukin-2 (IL-2) is captured and internalized by high affinity receptors on the cell surface. The Examiner then states that "therefore, the claimed invention could not be used to detect IL-2 secreting cells, because the IL-2 would have already been captured and removed from the cell surface." This conclusion is

not based on the findings of Weissman, but is an assertion made by the Examiner without any scientific basis in the art. If the Examiner is aware of any pertinent art to support this contention, or any scientific support, he is requested to provide this information in the form required by 37 C.F.R. §1.107(b).[1]

In fact, in contrast to the Examiner's assertion, the art indicates that IL-2 would be suitable for use in the claimed capture system. Weissman presents data showing that IL-2 is internalized by a high-affinity receptor on the surface of the human leukemia cell line HUT-102B2. Weissman also reports that there are two types of IL-2 receptors on the surface of these cells: a high- and a low-affinity receptor. The low-affinity receptor is not internalized to a significant degree (page 1464, Figure 1; and page 1465, column 2, paragraph 1). The presence of the high-affinity receptor, which internalizes IL-2, does not preclude the binding of IL-2 to the low-affinity receptor. The low-affinity receptor undergoes little or no internalization, even though the dissociation constants of the two receptors are quite different. A careful reading of the Weissman reference shows that the cells in question are capable of binding IL-2 on the surface in spite of the presence of a receptor that is internalized. Thus, there is every reason to believe that cells secreting a product which binds to a receptor capable of undergoing internalization can be used in the method or kit of the present invention. Since the Examiner has not presented evidence that the claimed method and kit would not work as described, the claims are supported by an enabling disclosure.

All the rejections on the grounds of enablement have been adequately addressed by the explanations presented above. Therefore, it is respectfully requested that these rejections under §112, first paragraph, be withdrawn.

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[1] 37 C.F.R. §1.107(b) requires that, "[w]hen a rejection in an application is based on facts within the personal knowledge of an employee of the Office, the data shall be as specific as possible, and the reference must be supported, when called for by the applicant, by the affidavit of such employee and such affidavit shall be subject to contradiction or explanation by the affidavits of the applicant and other persons."

Rejections under 35 U.S.C. §112, second paragraph

Claims 5, 6, 17, 19, 20, 41 and 51 are rejected under 35 U.S.C. §112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

Specifically, the Examiner states that claims 5 and 6 lack antecedent basis in claim 1 with respect to the recitation of "label moiety". Accordingly, applicants have amended claims 5 and 6 to correct claim dependency.

The Examiner further states that claims 17, 19 and 20 lack antecedent basis in claim 14 with respect to recitation of "specific binding partner". Claims 17, 19 and 20 have been amended to recite "capture moiety", which Applicants believe will overcome the rejection.

The Examiner states that claim 41 lacks antecedent basis in claim 34 in the recitation of "incubation conditions", and that claim 41 is indefinite in the recitation of "high viscosity or gel forming" because "it is unclear what this means or encompasses other than the specific examples recited in claim 42". The language of claim 41 has been modified such that claim 41 no longer relies on claim 34 for antecedent basis in recitation of "incubation conditions". Regarding the alleged indefiniteness in the recitation of "high viscosity or gel forming," although there is no evidence that this is, indeed, indefinite, Applicants have amended claim 41 to recite a " substance which slows diffusion of the secreted product from the producer cell". Support for this amendment is found on page 17, lines 12-29. Apparently, the Examiner would prefer that claim 41 be limited to the embodiments of claim 42, this is too stringent a requirement. There is no evidence that one of skill in the art would not be able to determine the appropriate viscosity conditions and materials with which to practice the invention. There is no requirement in patent law to restrict a claimed invention to actual enumerated embodiments.

The Examiner states that claim 51 is indefinite in the recitation of "cell-cell cross-contamination reducing capture system" because it is allegedly unclear what this means or encompasses. Applicants have amended claim 51 to recite a "trapping moiety"; support for this amendment is found on page 18, lines 9-32.

All the rejections on the grounds of indefiniteness have been adequately addressed by the explanations presented above and the amendments to the claims. Therefore, it is respectfully requested that these rejections under §112, second paragraph, be withdrawn.

Rejection under 35 U.S.C. §102(b)

Claims 1-3, 14, 15, 29 and 30 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Köhler and Shulman (1980) *Eur. J. Immunol.* 10:467-476 (hereinafter "Köhler").

Specifically, the Examiner states that Köhler teaches the cells and methods of claims 1, 14, 29 and 30 because "the product (e.g., IgM) is labeled with a label moiety (e.g., complement) prior to separation." Applicants respectfully traverse. In order to anticipate a claimed invention, the prior art must disclose every element of the invention. Richardson v. Suzuki Motor Co., 9USPQ2d 1920 (CAFC, 1989).

Köhler has been mischaracterized in the Office Action. Köhler does not disclose a *separation* procedure, but a *selection* procedure. The selection procedure disclosed in Köhler is furthermore a *negative* selection involving coupling the hapten trinitrophenyl (TNP) to the surface of cells which secrete antibody to the hapten, then incubating the cells in the presence of complement. Only mutants which either do not secrete antibody or secrete a mutated antibody incapable of binding hapten and/or fixing complement survive the treatment. Even after subjecting the cells to this negative selection procedure, Köhler discloses that the cells then had to be separated out by plating in methyl cellulose overlaying a layer of agarose containing protein A-coupled sheep red blood cells (SRBCs) and complement. This distinguishes those

cells that were still secreting antibody from cells which had survived initial selection merely because they no longer secreted antibody. Thus, not only is Köhler's technique a selection rather than a separation technique, but in order to obtain the desired population, *i.e.*, those that secrete mutated antibody, the separation technique used was the methylcellulose procedure, which of course is not the separation technique recited in claim 1.

Accordingly, claim 1 distinguishes over Köhler and need not be amended. Nevertheless, in the interest of expediting prosecution, Applicants have amended claim 1 to recite the limitation "wherein the labeled cells are not lysed as part of the separation procedure". Support for this limitation is found on page 5, lines 9-10 of the specification. This amendment suffices to overcome the rejection of claim 1 and of claims 2-3 and 29-30 which are dependent therefrom.

The Examiner states that in Köhler, the product IgM is labeled with a label moiety, i.e. complement, and therefore anticipates claim 14. Applicants traverse. As noted above, complement was not used as a label moiety. Rather, complement, when bound to IgM complexed with cell surface-coupled hapten, effected the lysis of those cells. A molecule that, when bound directly or indirectly to a cell surface molecule, effects cell labeling is clearly distinguished from one that, when bound directly or indirectly to a cell surface molecule, effects cell lysis. A lysed cell is clearly not a labeled cell; it is not a cell at all. Since complement is not a label moiety in Köhler, Köhler does not anticipate claim 14 or dependent claim 15.

Claims 22 and 23 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Weissman et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:1463-1466 (hereinafter "Weissman"). Specifically, the Examiner states that Weissman teaches "cells capable of capturing a product secreted and released by said cells wherein the surface of said cells is coupled to a specific binding partner for said product and wherein the specific binding partner is not a hapten and where the cells are coupled to said product (see abstract)." Note that Weissman was also cited by the Examiner in support of 35 U.S.C. §112, first paragraph, to support a contention that "the

claimed invention could not be used to detect IL-2 secreting cells" (Office Action, page 4, lines 27-28). This is in no way an acquiescence to the 35 U.S.C. §112, first paragraph rejection. It does, however, exemplify some of the differences between Weissman and the claimed invention, as acknowledged by the Examiner.

The Examiner asserts that claims 22 and 23 are clearly anticipated by Weissman. Applicants traverse. Weissman reports that a human T-cell leukemia cell line and a murine cytotoxic T-cell line express on their surfaces receptors with high-affinity binding sites for IL-2. The Examiner appears to be of the position that a receptor which the cell synthesizes and then incorporates into the cell surface is "coupled" by the cell to the surface. This use of the term "coupled" is not the art-accepted meaning. A receptor which is normally synthesized by a cell, inserted into its membrane and expressed on its surface is not considered in the art to be "coupled" to the cell. The accepted meaning of the term, in agreement with the disclosure of the present invention, is that a moiety is coupled to a cell not as a result of normal biosynthesis, but by an investigator.

Therefore, claim 22 is novel over Weissman. Nevertheless, in the interest of expediting prosecution, Applicants have amended claim 22 to read "A composition of matter which comprises cells capable of capturing a product secreted and released by the cells, wherein a capture moiety is anchored to the surface of the cells through an anchoring moiety, and wherein the capture moiety is not a hapten." Support for this amendment is found in the specification in the bridging sentence of page 9-10 and on page 10, paragraphs 1 and 2.

All the rejections under 35 U.S.C. §102(b) have been adequately addressed and the above discussions and amendments to the claims overcome the rejections. Therefore, it is respectfully requested that these rejections be withdrawn.

Rejections under 35 U.S.C. §103

Claims 1-30, 34-40, 43-50 and 53-56 are rejected under 35 U.S.C. §103 as allegedly being unpatentable over Köhler in view of Hunt, Chapter 55, from *Handbook of Experimental Immunology* Vol. 2, Eds. D.M. Weir et al., Blackwell Sci. 1986 (hereinafter "Hunt"), Segal (U.S. Patent 4,676,980) and prior art disclosed in the specification.

The Examiner alleges that it would have been obvious to one of ordinary skill in the art to combine the teachings of Köhler and Hunt to arrive at the present invention. Applicants traverse. The Examiner suggests that one of ordinary skill in the art would have combined the teachings of Köhler, which the Examiner states teaches a method for capturing a product secreted by a cell by labeling the cell with a specific binding partner which captures the secreted product, and Hunt, which the Examiner states teaches the use of cell sorting based on fluorochrome labeled antibodies. In order to establish a *prima facie* case of obviousness, there has to be some motivation or suggestion, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. The combined art does not satisfy this requirement.

Further, the Examiner consistently refers to the result of the combined references as showing what one of skill in the art "would" do.[2] Importantly, there is no evidence presented that, absent the teachings of the present specification, one of skill in the art would perform any of these steps and, if so, whether they would expect it to succeed. At best, this line of reasoning

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[2] "A routineer would have simply labeled the molecule captured by Köhler et al...." (page 6, lines 23-24); "[a] routineer would have used bispecific antibodies which bind any art known molecule..." (page 7, lines 2-3); "[a] routineer would have used any desired capturing probe instead of TNP..." (page 7, lines 6-7); "[a] routineer would have prepared a kit containing reagents necessary to practice..." (page 7, lines 14-15); and "[t]he kit would have included a high viscosity or gel forming medium..." (page 7, lines 15-16).

could support only an obvious to try rejection[3], at worst, it supports only a hindsight rejection.[4] Neither of these supports a finding of unpatentability under 35 USC §103.

The Examiner notes that the rejected claims are drawn to a method to separate labeled cells according to a product secreted and released, cells produced by said method and a kit. As noted above in the response to the rejection under 35 U.S.C. §102(b), Köhler does not teach a separation technique, but a negative selection technique. The separation technique disclosed in Köhler to isolate the desired cells is a cloning technique and is described on page 469, section 2.9, paragraph 1, wherein the selected cells are cloned in methylcellulose overlaying agarose containing complement plus Protein A-coupled sheep red blood cells (SRBC's). The desired cells, *i.e.*, ones secreting IgM, were detected by their ability to lyse the Protein A-coupled SRBC's and were visualized by plaque formation. Furthermore, Köhler teaches away from the use of fluorescence-activated cell sorting (FACS) as a separation technique. Köhler describes attempts to enrich for mutants that secrete IgM using FACS and concludes that "the FACS sorting led to no enrichment for IgM-secreting cells" (bridging paragraph of pages 469-470). It is improper to combine references where one reference teaches away from their combination. In re Grasselli, 218 USPQ 769, 779 (Fed. Cir. 1983).

The problem of separating cells based on a secreted product was not solved by Köhler. Indeed, the problem of separating cells on the basis of a secreted product was not even addressed by Köhler. Köhler teaches a negative selection technique, discloses that to separate or isolate the clones, standard techniques of methylcellulose or soft agar cloning were used, and states that

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[3] "In some cases, what would have been 'obvious to try' would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful." In re O'Farrell, 7 USPQ 2d 1673, 1681 (CAFC 1988). (Citations omitted.)

[4] "It is impermissible, however, to engage in a hindsight reconstruction of the claimed invention, using the applicants' structure as a template and selecting elements from references to fill the gaps." In re Gorman, 18 USPQ2d 1885 (Fed. Cir. 1991).

FACS led to no enrichment for secreting cells. The use of FACS was known at the time of the invention and was used to separate populations of cells based on surface markers, but the solution to the problem of separating cells based on a secreted product generally relied on the much more cumbersome techniques described by Hunt. The combination of these two references could not have yielded the method, cells and kit of the present invention. Therefore, the first criterion for establishing a *prima facie* case of obviousness has not been met.

The second criterion for a *prima facie* case of obviousness is that there must be a reasonable expectation of success. Again, since Köhler does not disclose the use of product coupled to a cell surface for the purposes of separation and actually teaches away from the use of FACS, the second criterion has not been met. The initial burden is on the Examiner to establish that there is some suggestion of the desirability of doing what the inventor has done. Either the references must expressly or impliedly suggest what the inventor has claimed, or the Examiner must present a convincing line of reasoning why the invention is obvious. MPEP 2142. Once a case of *prima facie* obviousness is established, the burden shifts to the inventors to provide rebuttal evidence. In this case, *prima facie* obviousness has not been established, thus it is not incumbent on the inventors to provide additional evidence of patentability. Indeed, since a combination of the references does not teach or suggest the claimed invention, it would not be technically possible to provide comparison data.

The Examiner goes on to cite Segal. Segal teaches that bispecific antibodies can bind a cell surface antigen and a second antigen, thus bringing the second antigen into proximity of the cell surface. The Examiner contends that "[a] routineer would [have] used bispecific antibodies which bind any art known molecule that exists on the surface of a desired target cell." The Examiner presumably cites Segal with respect to claim 35, which as amended recites a kit for use in the detection of cell that secrete a desired product, the kit comprising at least one bispecific antibody having at least one antigen recognition site for at least one cell surface structure and at

least one antigen recognition site specific for a secreted product, and at least one label moiety." Segal does not teach the use of bispecific antibodies to capture secreted product. Segal teaches bispecific antibodies wherein one antibody combining site is specific for a cell surface molecule on a cytotoxic effector cell and a second antibody combining site is specific for a cell surface molecule on a target cell; such that the bispecific antibody brings the two cells in proximity to one another, the desired outcome being killing of the target cell by the cytotoxic effector cell. Nowhere in Segal is it suggested to use a bispecific antibody in which one antibody combining site is specific for a secreted product. Therefore, Segal does not render the present invention obvious.

The Examiner further states, apparently by way of connecting the teachings of Köhler with those of Segal, that Köhler teaches that TNP is connected to the cell surface via an antibody. Careful reading of Köhler shows that TNP is in fact covalently coupled to the cell surface by reacting the cells with 2,4,6-trinitrobenzene sulfonic acid (TNBS) (page 469, section 2.8 paragraph 2, lines 1-4). Therefore, on yet another ground, Köhler combined with Segal does not suggest the present invention, since Köhler does not teach coupling a capture moiety to the cell surface via an antibody and Segal does not teach the use of a bispecific antibody to capture a secreted product. Neither reference suggests a positive selection procedure and thus cannot be said to teach or suggest even the general area of the claimed invention.

All the rejections under 35 U.S.C. §103 have been adequately addressed and the above explanations and amendments to the claims overcome the rejections. Therefore, it is respectfully requested that these rejections be withdrawn.

III. CONCLUSION

Applicants submit that the above discussion is fully responsive to all grounds of objection and rejection set forth in the Office Action. In view of the comments above, Applicants

respectfully request that all outstanding rejections be withdrawn, and that the pending claims, as amended, be allowed.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (650) 813-5695.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952**. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

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Respectfully submitted,

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